Aflatoxin B1 (AFB1) has been recognized to produce cancer in human liver. In addition, epidemiological and laboratory studies demonstrated that the respiratory system was a target for AFB1. Exposure occurs predominantly through the food chain, but inhalation represents an additional route of exposure. The present study aimed to examine AFB1 exposure among poultry workers in Portugal. Blood samples were collected from a total of 31 poultry workers from six poultry farms. In addition, a control group (n = 30) was included comprised of workers who undertook administrative tasks. Measurement of AFB1 in serum was performed by enzyme-linked immunosorbent assay (ELISA). For examining fungi contamination, air samples were collected through an impaction method. Air sampling was obtained in pavilion interior and outside the premises, since this was the place regarded as the reference location. Using molecular methods, toxicogenic strains (aflatoxin-producing) were investigated within the group of species belonging to Aspergillus flavus complex. Eighteen poultry workers (59%) had detectable levels of AFB1 with values ranging from <1 ng/ml to 4.23 ng/ml and with a mean value of 2 ± 0.98 ng/ml. AFB1 was not detected in the serum sampled from any of the controls. Aspergillus flavus was the fungal species third most frequently found in the indoor air samples analyzed (7.2%) and was the most frequently isolated species in air samples containing only Aspergillus genus (74.5%). The presence of aflatoxigenic strains was only confirmed in outdoor air samples from one of the units, indicating the presence of a source inside the building in at least one case. Data indicate that AFB1 inhalation represents an additional risk in this occupational setting that needs to be recognized, assessed, and prevented.